Innovative methods of ground improvement for railway embankment Peat Fens foundation soil

Safdar UM[1], Mavroulidou M[1]*, Gunn MJ[1], Garelick J[2], Payne I[3], Purchase D[4]

Abstract

The aim of this research was to assess the feasibility of biocementing a problematic foundation soil of railway embankments from Peat Fens in East Anglia, UK. Biocementation of soil is an emerging, novel ground improvement technique. It has recently attracted the interest of researchers worldwide because it has been proposed as potentially environmentally superior to chemical grouts and other common soil stabilisers e.g. cement or lime (linked to high CO_2 11 emissions). In this study we screened and isolated non-pathogenic indigenous ureolytic microbial candidates with potential for biocementation from samples originating from Peat Fens in East Anglia, UK. Four strains were selected as the most suitable candidates, based on their growth rate and their viability in a wide range of temperatures, pH and soil moisture contents corresponding to typical seasonal field conditions. After a number of Unconfined Compressive Strength (UCS) tests, one strain (*Bacillus licheniformis*) was selected as the most promising for this soil treatment and used for further study. Two different methods of implementation of the treatments were considered, namely pressure flow soil column and electrokinetic injection. The UCS results supported by $CaCO_3$ measurements as well as microstructural SEM-EDS analysis proved that biocementation did occur for both implementation methods and for a number of treatment combinations. Ongoing work on optimisation of treatments and implementation methods is carried out towards the upscaling of the techniques for in situ implementation which is planned for the next stage of the research.

Keywords: Ground improvement; Organic soils; Electrokinetics

1 Introduction

Emerging challenges for geotechnical engineers who develop or maintain linear transport infrastructure are currently greater than ever before: new, complex transport infrastructure, with little tolerance for error (e.g. high-speed trains) will be increasingly constructed on areas of inferior ground due to the scarcity of urban space. Existing infrastructure facilities will also need to be upgraded to meet future needs and changing environmental loads due to climate change.

[1] Division of Civil and Building Services Engineering, London South Bank University, UK

[2] Design Delivery, Network Rail, UK

[3] Geotechnical Asset Management, East Anglia Route, Network Rail, UK

[4] School of Natural Sciences, Faculty of Science and Technology, Middlesex University, UK

These include ageing transport earthworks in many European countries suffering from serviceability problems and requiring costly maintenance/ remediation. This causes a major constraint for infrastructure owners and operators, especially in the light of the increased risk of hazards posed by climate change.

Government policies require infrastructure to be provided in an economical and environmentally responsible manner. Thus, improving rather than replacing and landfilling inferior geomaterials, will become critically important in future engineering practice towards low-carbon solutions (Simpson and Tatsuoka, 2008). Unfortunately, common ground improvement methods suffer from high costs, disturbance/interruption of services and environmental side-effects. There is therefore an urgent need for superior, innovative techniques and practices for ground improvement. In line with this, an emerging technique, which has recently attracted the interest of researchers worldwide, is soil biocementation. It utilises the natural biological process of biomineralisation (the biological production of minerals through the metabolic processes of different types of microorganisms/ plants) as a soil stabilisation method. The technique has been proposed to be environmentally superior to chemical grouts (which can be potentially toxic/hazardous - see e.g. Karol, 2003 -, unlike microbially produced grouts) or other common soil stabilisers e.g. cement or lime (linked to high CO_2 emissions) and potentially more sustainable overall, because the micro-organisms are natural, readily available and renewable and those proposed for in situ uses are non-pathogenic (DeJong et al, 2013).

The use of biomineralisation for other in-situ applications such as water treatment or contaminated ground bioremediation is well established (Arias et al, 2017; Chen et al, 2015). However, the use of biomineralisation as a soil stabilisation technique has been less explored. The most widely investigated process has been calcium carbonate precipitation to biocement sand using urea hydrolysing bacteria which, via the catalytic action of urease enzyme, hydrolyse urea into ammonia (NH_3) and carbonic acid (H_2CO_3) (see reaction (1)). The products equilibrate in water to give bicarbonate (HCO_3^-), ammonium (NH_4^+) and hydroxyl ions (OH^-), respectively (see reactions (2) and (3)). The formation of the hydroxyl ions (OH^-) from reaction (3) leads to an increase in pH, which in turn leads to the formation of carbonate ions (CO_3^{2-}) (see reaction (4)). In the presence of dissolved calcium ions (Ca^{2+}) the carbonate ions (CO_3^{2-}) thus produced react with calcium ions (Ca^{2+}) and precipitate as calcium carbonate ($CaCO_3$)

$$CO(NH_2)_2 + 2\,H_2O \xrightarrow{\text{Urease}} 2\,NH_3 + H_2CO_3 \quad (1)$$

$$H_2CO_3 \rightleftharpoons HCO_3^- + H^+ \quad (2)$$

$$NH_3 + H_2O \rightleftharpoons NH_4^+ + OH^- \quad (3)$$

$$HCO_3^- + OH^- \rightleftharpoons CO_3^{2-} + H_2O \quad (4)$$

$$Ca^{2+} + CO_3^{2-} \rightarrow CaCO_3\downarrow \quad (5)$$

$CaCO_3$ crystals then cement soil particles together, to increase soil strength and stiffness. Following the Australian researchers (Whiffin, 2004; Al Thawadi, 2008), the vast majority of subsequent studies used the same ureolytic bacterium, *Sporosarcina pasteurii,* proven to be effective for biocementation (e.g. Al Qabany et al, 2012; Montoya et al, 2013; Montoya and De Jong, 2015; Gao et al 2018 amongst many others). Few works extended the technique different soils using *Sporosarcina pasteurii*, e.g. van der Star et al (2011): gravel; Rebata-Landa (2007) and Mortensen et al (2011):silt; Ng et al (2014): a residual silt soil; and Mavroulidou et al, (2011): silt and silt-clay mixes (in this study *Sporosarcina ureae* was also used).

Most biocementation studies used the process of bioaugmentation i.e. the supply of precultured microorganisms into the soil to enhance microorganism populations at a site. The alternative process of biostimulation, where nutrients can be added in the ground to stimulate native micro-organism growth was applied in a limited amount of studies only: for instance, indigenous urea-hydrolysing bacteria were used in works by Sato et al (2016) and Danjo and Kawasaki (2016), who treated peat and created artificial beachrocks against coastal erosion activity in laboratory conditions respectively or in Gomez et al (2017) who used biostimulation in pilot in situ applications of sand treatment.

Biocementation applicability to fine grained soils has been contested because the size of an individual bacterium is similar or larger than the largest size of clay particles. Small pore and pore throat size (recommended to be not less than 0.4 μm in Mitchell and Santamarina, 2005) would restrict the transport and growth of bacteria as well as air, water and substrate fluxes, thus influencing microbial activity (Or et al, 2007; Rebata-Landa, 2007; Negassa et al, 2015).

Keykha et al (2014) and Keykha et al (2018) thus used an alternative approach injecting electrokinetically into the fine-grained soil externally produced CO_3^{2-} through bacterial action (according to (4)) but not the bacteria themselves. In electrokinetics (EK) an electric current applied within the porous media to induce specific transport phenomena. As an implementation technique for fine-grained soils and in particular under existing infrastructure EK can be very useful in (a) effectively delivering chemicals or nutrients to indigenous bacteria in the soil for biostimulation; (b) introducing bacteria into the soil while enhancing bioavailability of treatments and (c) giving a more uniform flow distribution and control over the flow direction. EK has been mainly used as a contaminant remediation technology (also combined with bioremediation but to a lesser extent, e.g. Lageman et al, 2007; Barba et al 2018); conversely using EK to convey treatments for geotechnical applications is rare in particular if combined with biocementation (with the exception of laboratory studies by Keykha et al, 2014 and 2018).

The aim of this research was to assess the feasibility of biocementing soil samples from Peat Fens (East Anglia railway network route, UK) with the perspective that, if proved in the laboratory, biocementation could then be tried as a suitable ground improvement method in situ.

Peat Fens constitute a soft, unstable foundation soil of East Anglia railway embankments subject to settlements due to a number of possible causes (consolidation, creep, oxidation and wastage as well as shrinkage, upon lowering of the water table: Hobbs, 1986). The research question of this study is thus whether Peat Fens soil can be biocemented using indigenous microorganisms. The hypothesis

is that biocementation would lead to an increase in the strength of the soil accompanied by an increase in its calcium carbonate content. The hypothesis will be tested through unconfined compressive strength (UCS) measurement, supported by chemical and microstructural analysis. Unlike most other biocementation studies here we aim to biocement a natural soil matrix of approximately 51% of organic content. As opposed to the vast majority of other works, here indigenous microorganisms were isolated and screened to be used for biocementation of the same soil. Finally, another novelty is the EK injection of the treatments, combining biocementation and EK processes, as a potentially suitable method for the implementation of treatments under existing embankments.

2 Materials, methods and processes

2.1 Soil sample characteristics

The soil used in this study (provided by Network Rail) was taken from two boreholes at a site of the East Anglia railway network route. The 8 m samples were divided into 8 equal parts.

Duplicate samples were tested to establish the physico-chemical characteristics of each layer and for bacterial strain isolation. However for this paper, the results are based only on samples of natural soil from depths of 0-2 m with similar pH, moisture and organic matter contents for consistency (see Table 1). In its as-received state the natural soil was of very dark greyish brown colour (10YR 3/2 according to Munsell chart) and consisted of a mixture of mineral and organic soil fractions. Although looking solely at the particle size distribution the soil would be classified as silty sand (or sandy loam according to USDA soil taxonomy, USDA 1999) due to its organic content, which was greater than 20%, it was identified as amorphous peat (i.e. “of no visible plant structure and mushy consistency”, according to the BS EN ISO 14688-1:2018 (BSI, 2018). This was consistent with its low natural moisture content shown in Table 1 (the more humified /decomposed the organic soil is, the smaller the water content). Based on its ash content by dry weight which is < 25% the soil is equally classified as peat according to ASTM D4427-92 (1997) (namely: basic sapric peat). The sample was pulverised with a rubber pestle. As the soil came from a shallow depth it was sieved to remove inorganic debris. Thus only the portion passing the 1.18 mm sieve was retained for further testing. Based on sieving followed by hydrometer testing (according to BS 1377:1990) this portion consisted of approximately 74% sand–sized, 20% silt-sized and about 6% clay-sized particles (see Fig 1). Other basic characteristics of this sample portion are shown in Table 1 (natural gravimetric moisture content and Atterberg limits in Table 1 were obtained from tests on the wet soil -i.e. not oven-dried- and expressed on a dry soil mass basis -corrected for moisture of the sample). Note that it was possible to determine the plastic limit of the soil. Hobbs (1986) suggested that this information is a useful morphology indicator as this test is possible for fen peats and transitional peats but not for bog peats unless almost completely humified.

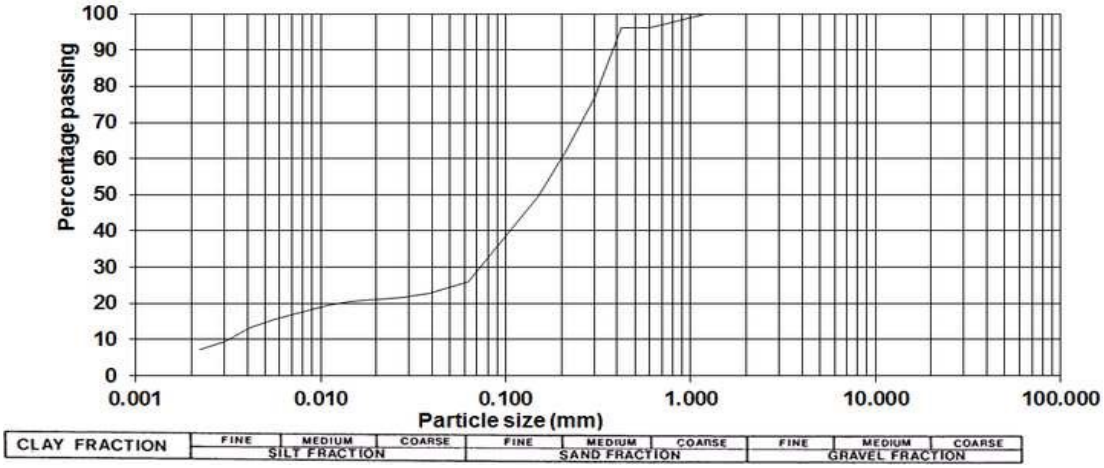

Fig. 1. Particle size distribution of retained soil sample portion (passing 1.18 mm sieve)

Table 1. Basic physicochemical properties of soil sample

Property	Value	Test/Standard
Liquid limit (% w/w)	101	Cone penetrometer; BS 1377 : Part 2 : 1990
Plastic limit (% w/w)	63	BS 1377 : Part 2 : 1990 (BSI, 1990)
Plasticity index (% w/w)	38	BS 1377 : Part 2 : 1990 (BSI, 1990)
Specific gravity, G_s	2.06	BS EN ISO 11508:2017 (BSI, 2017)
Natural gravimetric moisture content (% w/w)	55.5	BS EN ISO 17892 : Part 1 : 2014
pH (of soil suspended in distilled water)	7.15	BS ISO 10390:2005 (BSI, 2005)
Ash content (% w/w)	17.7	ASTM D2974-14 (ASTM, 2014)
Organic matter content (% w/w)	50.8	Loss of ignition; ASTM D2974-14 (ASTM, 2014)

2.2 Microbiological study

2.2.1 Enrichment for ureolytic bacteria and isolate selection

Isolation of bacteria was done by using 1 g of soil from each soil sample diluted in sterile distilled water at the required dilution. Note that saline Ringer solution could have been alternatively used. However, this would have made very little difference as in the natural soil sample, which is rich in in nutrients and ions, the osmotic pressure in the diluted soil sample would not have been affected; furthermore, the diluted soil solution was immediately plated out onto the agar plate. Namely, 1 mL of the diluted culture was plated out on 15 mL of Tryptic Soya Agar (TSA, Oxoid, UK), incubated at 25°C for 3-7 days. Samples that showed considerable growth at either 25 or 37°C (based on plate counting) were transferred to B4 Agar plates. B4 medium (pH 7.3) consists of 0.4 % yeast extract, 0.5 % dextrose, 0.25 % calcium acetate and 1.4% agar in solid preparations (Bouquet et al, 1973). It has been used since the seventies (Boquet et al, 1973) and has been the preferred medium for s 142 tudying mineral precipitation (and in particular $CaCO_3$) *in vitro* using bacterial strains isolated from different ecosystem environments (Marvasi et al, 2012). The strains were then incubated at 37°C for one week to form crystals. Colonies that showed good production of crystals as confirmed microscopically were selected and passaged twice on B4 plate to obtain purified single colonies. The selected strains were transferred to Nutrient Agar and Nutrient Broth (Oxoid, UK) for storage at 4°C and -80°C respectively. For the geotechnical analyses, all the test strains were cultivated at pH 7 under aerobic batch conditions in a sterile culture medium of Nutrient Broth (Oxoid, UK) consisting of 5-g/L peptone, 5-g/L sodium chloride, 2-g/L yeast extract, and 1-g/L beef extract.

Incubation was performed in a shaking incubator at 200 rpm and 37°C. The strains were grown to an early stationary phase i.e., Optical Density (OD): OD600 ranging from 0.5-0.7 (measured using a Pharmacia LKB Novaspec II spectrophotometer of a Wave length Range 325-900 nm); they were then harvested by centrifuging at 8000 g for 10 minutes to achieve the final concentration of approximately $1x10^8$ cfu/mL (optical density 3.3); a second concentration of $1x10^7$ cfu/mL was obtained by dilution with sterile sodium chloride solution (9 g/L NaCl).

2.2.2 Microbial identification and diagnosis

Microbial identification and diagnosis were performed using matrix-assisted laser
160 desorption/ionization time-of-flight/time-of-flight tandem mass spectrometry (MALDI-TOF/TOF MS) proteomic-based biotyping approach (Singhal et al, 2015). The sample preparation and extraction of proteins and peptides of the bacteria were performed according to the Bruker bacterial sample preparation protocol. Each extracted sample was analysed using a MALDI ground steel plate and six different sample spots (replicates) to generate six combined mass spectra (MSP) per bacterial isolate. The acquisition and analysis of mass spectra were performed by MALDI-TOF/TOF MS. The identification of the isolated bacteria strain through comparison with reference strains and visualization of the mass spectra was performed with MALDI Biotyper software 3.0 (Bruker Daltonics).

2.3 Chemical analyses

2.3.1 Urease activity

Urease activity directly controls the rate of urea hydrolysis and as such the calcium carbonate
173 concentration; it is therefore a key factor governing the bio-cementation process when using ureolytic bacteria. The urease activity and the resulting ammonia concentration in the treated soil was directly measured by using Urease Activity Assay kit (Colorimetric; Abcam, US) using the following steps: Clear supernatant containing urease was obtained by centrifugation for 5 min at 14,000 x g for each bacterial species. The enzyme reaction was performed at pH 7 at 30°C for 4 hours using the following steps: 0.1 mL supernatant was collected and added in a micro vial. For the test sample, 0.1 mL of Urea was added into 0.9 mL solution tube and incubated at 37°C for 2 hours. The solution was then centrifuged at 8000 x g for 1 minute. 0.1 mL supernatant was collected and then added in a micro vial in which reagents were added and vortexed with mechanical mixer. The solution was again incubated at 37°C for 30 minutes. The output was measured on an ultraviolet-visible spectrophotometer at OD670. At the end of the reaction, strains that produced < 30 µm of ammonia were shortlisted.

2.3.2 Calcium carbonate ($CaCO_3$) content

$CaCO_3$ content of the treated samples was determined by acid digestion test using 20 g of oven-dried (at 105 °C) soil samples soaked with 2 M hydrochloric acid (HCl) (Ng et al, 2014). The presence of carbonate was manifest as samples fizzed vigorously. The residue was collected on a filter paper and oven dried at 105 °C and the mass loss measured to estimate the calcium carbonate content in the soil expressed as a percentage of the dry sample mass (i.e., 20 g).

2.4 Treatments and implementation methods

2.4.1 Tested samples

A number of control samples and samples with treatments with or without bacteria were prepared. The list of samples presented in this paper is summarised in Table 2, together with the respective implementation methods. Namely, in addition to testing the untreated soil statically recompacted at its original dry density of 0.919 g/cm_3 (see Table 2, Test ID: 'Natural'), three additional sets of control samples were prepared (in triplicate) as follows: 1. For the pressure flow column: (a) untreated soil samples with their moisture content adjusted by adding15 % of water per dry soil mass, consistent with the increased 202 moisture content of the treated samples due to the addition of treatment solutions (PFC1); (b) soil samples treated with 15% per dry soil mass nutrient broth solution (of a 3 g/L concentration) (PFC2) and (c) soil samples with added nutrients and bacteria only (no cementing agents), to study possible effects of microorganism cells on soil strength (PFC3-6). Two sets of treated samples for all four bacteria were prepared at two different bacteria populations and cementing agent dosages (PFC9-16).

From these tests, the best strain was selected for further parametric study, which is ongoing (investigating treatment dosages and curing time effects); selected results of this study are included here (PFC7-8).

2.4.2 Soil column experimental setup

Figure 2 shows the experimental setup for the Microbially-Induced Calcite Precipitation (MICP) treatment according to the first implementation method, i.e. pressure driven flow through a soil column (referred to herein as "pressure flow column"). The apparatus consisted of a Plexiglas cylindrical mould (50 mm in diameter and 170 mm in length), a hydraulic pump, a compression frame and an effluent collector. The inner surface of the Plexiglas mould was coated with a non-reactive lubricant for easier specimen extraction after the treatment. To prepare the soil specimens, soil passing the 1.18 mm was prepared. For the first series of investigations (presented in this paper) the soil was first mixed with the individual microorganisms (in the case of bio-augmentation) before the addition of the treatment solutions (nutrients and cementing agents, as applicable). We did this to investigate biocementation feasibility with a simpler method and more likely to supply the bacteria uniformly into the soil, hence surmounting one possible reason for unsatisfactory MICP results (once MICP feasibility has been proven as a next step implementation of bacteria into the soil by methods other than mixing will be investigated). Cementing reagents were equimolar urea $(CO(NH)_2)_2$ and calcium chloride ($CaCl_2$) solutions (according to stoichiometry). Note however that often a urea:calcium ratio of 1.25:1 to 3:1 may be used to provide additional urea to be hydrolysed to further increase the pH (Ivanov et al, 2019). Urea was used as an energy and ammonium source for the hydrolysis process described in (1) (Mitchell and Santamarina 2005) and calcium chloride was used as a calcium source (5). To identify best concentrations for strength development for the studied soil, we used solutions of different molarities in the range of those used in previous MICP works for other soils (e.g. Whiffin et al, 2007; Al-Qabany and Soga, 2013; Ng et al 2014 amongst many others). Sufficient water for the control sample or aqueous solution of Nutrient Broth for the biocementation samples of a total of 15 % by mass of the soil sample was then supplied (this added percentage was kept consistent throughout all the samples during the MICP). The soil was then covered with air tight seal and left for 48-72 hours to attain homogeneity of treatments throughout the specimen. Standard UCS specimens were then made from the soil sample, by static compaction at a rate of 1mm/min to a dry density of 0.919 g/cm$_3$ and were transferred into the Plexiglas mould. The soil specimen was sandwiched between the two layers of perforated disks and filter papers, to avoid turbulent inflow and clogging at the inlet and outlet and mounted tightly onto the compression frame. The mould inlet was connected to the outlet of the pump.

The cementing reagent solution was supplied into the specimen mould at a constant flow pressure of 150 kPa by regulating the pressure from the pump's control panel and at room temperature (22-27°C). The applied pressure value was selected after some preliminary trial tests and considering the equipment limitations (pump able to provide up to 200 kPa pressure).

Pressures over 150 kPa damaged the sample (also resulted in clogging of the tubing as a consequence of this); pressures below 90 kPa resulted into too slow flow rates and uneven treatment distribution (higher at the top than at the bottom of the sample, presumably due to clogging of the pore

throats close to the injection points, a known problem discussed in the literature, see e.g. Whiffin et al. 2007). The pH of the effluent was monitored by sampling the effluent from the specimen mould at 24-h intervals; ammonium measurements in the effluent (at 24-h intervals) as well as in the pore water of the treated soil specimens (at the end of curing period) were measured with the Urease Activity Assay kit (Colorimetric; Abcam, US) using a modified Berthelot method.

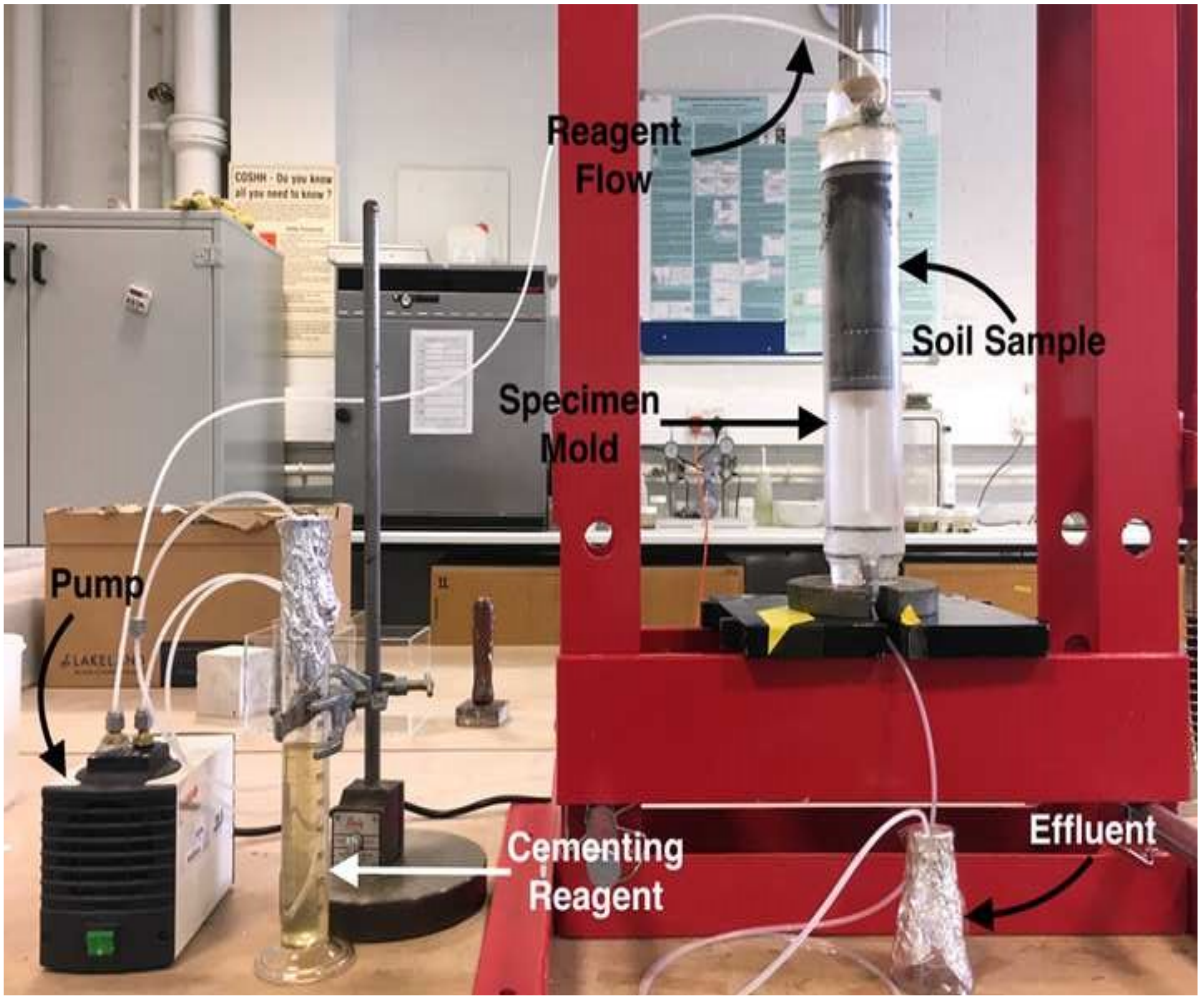

Fig. 2. Pressure flow column set up

2.4.3 Electrokinetic (EK) experiments

EK cell designed for this study was a tank of internal dimensions 210 mm length x 160 mm width x 140 mm depth after the incorporation of a purpose- built sample extractor internal layer to prevent sample disturbance during extraction at the 262 end of the test. The tank was made of 10 mm thick nonconductive material (transparent acrylic 'Perpex' sheet) and had perforated partition walls (perforated area 40 % of total wall area) between the soil containing chamber and the electrolyte chambers of internal dimensions 100 mm length x 160 mm width x 140 mm depth (see Fig. 3). A layer of filter paper was used on the perforated walls to prevent the movement of soil particles into the electrolyte chambers. Inert graphite sheet electrodes of 99 % purity were used to eliminate electrode corrosion that would introduce secondary reaction products and reduce the effectiveness of the system

due to substantial voltage loss at the electrodes. The soil sample was compacted using a hydraulic compression frame in 5 equal layers to the required dry density of 0.919 g/cm3 of the undisturbed soil. A constant voltage gradient of 0.4 V/cm was maintained throughout the tests as recommended in the literature in order to prevent potential harm to the bacteria (Mizuno and Hori, 1988; Hassan et al, 2016). For the same reasons, periodic polarity reversal was applied every 24 h recommended for a better uniformity hence effectiveness of the treatment but also to prevent high pH gradients that could also be harmful to the bacteria (Mena et al, 2016). To ensure that both voltage and pH did not considerably change during treatment, measurements of these quantities were performed at 0 mm, 50 mm and 100 mm away from the electrodes locations of the sample throughout the treatment; pH of the solutions was also monitored inside both electrolyte chambers during the treatment. In addition, temperature was recorded as this can affect bacteria growth. The dimensions of the cell allowed for the extraction of duplicate UCS specimens (50 mm diameter and 100 mm height cylinders) from three different locations in the soil sample, namely from the areas next to the two electrolyte chambers and from the middle of the sample. Before starting the EK biocementation testing plan, the following preliminary investigatory tests were performed: (a) a test starting from the natural moisture content of the soil, where soil was let to dry out due to electroosmosis (without adding any water at the anode compartment); this showed a 13.6 % change in the volume of the soil; (b) making the simplifying assumption that the soil was saturated, the 13.6 % volume change of the soil upon EK was compensated by adding 13.6 % at the anode compartment in an attempt to maintain a constant soil volume during EK testing; this is of practical importance to avoid soil settlement under existing earthworks during EK treatment. The results showed that this water addition had successfully prevented volume change of the soil during EK. Therefore, the addition of 15 % water (or aqueous 292 solutions, with the water content being reduced approximately up to 14.59 % if the molecular weights of the nutrient broth, urea and calcium chloride are considered) consistent with the pressure flow column tests, was considered acceptable to maintain a relatively constant sample volume during the EK treatment and was adopted for the rest of the tests with bacteria implementation. The nutrient broth solution and the cementing reagents were supplied all in one single solution (divided equally in the two electrolyte compartments i.e.7.5% per dry soil mass per compartment). The EK treatment lasted for two weeks, (i.e. 7 days per electrode polarity) which is a typical field treatment length (e.g. Mena et al, 2016) and was used here to prove the biocementation feasibility. However, in further tests 7-day duration treatment will also be attempted for direct comparisons with the pressure flow tests. Note that in the presented test EK2 the bacteria were mixed with the soil before the application of the EK treatment used to supply the nutrients and cementing reagents. The reason for this was to prove the feasibility of the technique initially having to consider only the potential effect of the EK on bacteria as an influential factor on the biocementation success, thus eliminating any other complications linked to the bacteria transport and distribution in the sample if supplied electrokinetically. However, in further planned tests the bacteria are also supplied electrokinetically consistently with the realistic field implementation of the treatment.

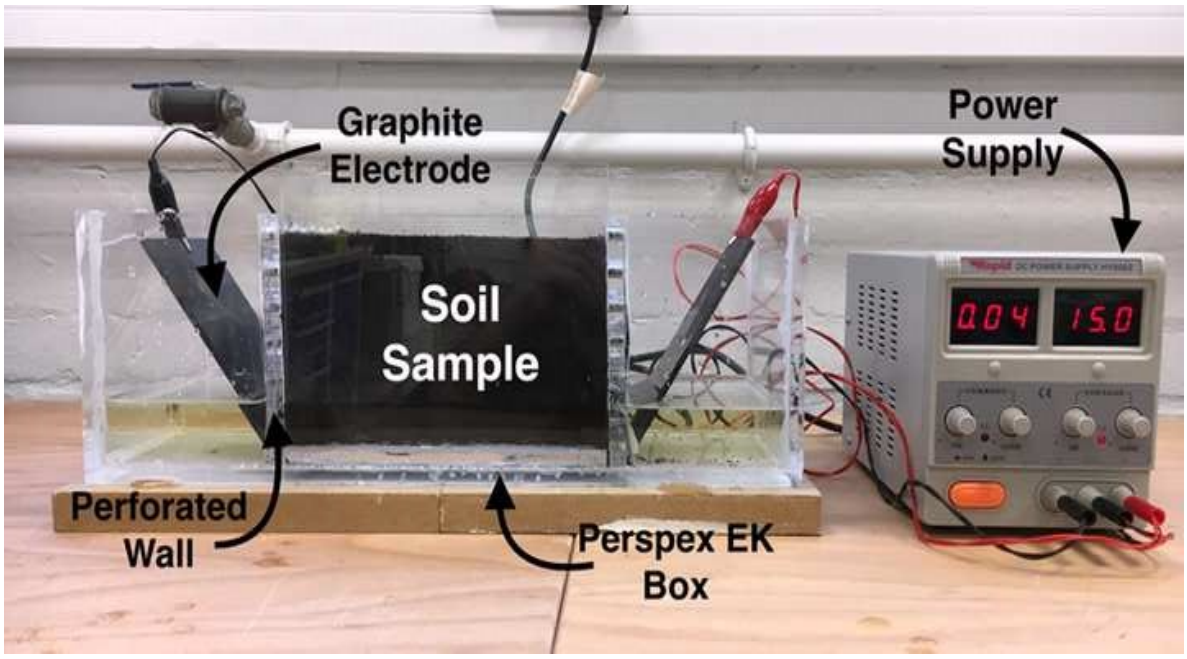

Fig. 3. Electrokinetic system set up

Table 2: List of UCS testing specimens

Test ID	Implementation	Added solutions	Added Strain	Population of added culture (cfu/mL)	Cementing reagent concentration CH_4N_2O (M) : $CaCl_2$ (M)	Treatment duration (days)	Curing (days)
Natural	N/A	N/A	N/A	N/A	N/A	N/A	N/A
PFC1	Pressure Flow Column	N/A (distilled water)	N/A	N/A	N/A	3	7
PFC2	Pressure Flow Column	3g/L nutrients	N/A	N/A	N/A	3	7
PFC3	Pressure Flow Column	3g/L nutrients	*Bacillus licheniformis*	$1x10^8$	N/A	3	7
PFC4	Pressure Flow Column	3g/L nutrients	*Rhodococcus erythropolis*	$1x10^8$	N/A	3	7
PFC5	Pressure Flow Column	3g/L nutrients	*Micrococcus luteus*	$1x10^8$	N/A	3	7
PFC6	Pressure Flow Column	3g/L nutrients	*Lysinibacillus fusiformis*	$1x10^8$	N/A	3	7
PFC7	Pressure Flow Column	3g/L nutrients + cement. reagent	*Bacillus licheniformis*	$1x10^8$	0.25:0.25	3	1
PFC8	Pressure Flow Column	3g/L nutrients + cement. reagent	*Bacillus licheniformis*	$1x10^8$	0.75:0.75	3	1
PFC9	Pressure Flow	3g/L nutrients +	*Bacillus*	$1x10^8$	1:1	3	7
PFC10	Pressure Flow Column	3g/L nutrients + cement. reagent	*Rhodococcus erythropolis*	$1x10^8$	1:1	3	7
PFC11	Pressure Flow Column	3g/L nutrients + cement. reagent	*Micrococcus luteus*	$1x10^8$	1:1	3	7
PFC12	Pressure Flow Column	3g/L nutrients + cement. reagent	*Lysinibacillus fusiformis*	$1x10^8$	1:1	3	7
PFC13	Pressure Flow Column	3g/L nutrients + cement. reagent	*Bacillus licheniformis*	$1x10^7$	0.5:0.5	3	7
PFC14	Pressure Flow Column	3g/L nutrients + cement. reagent	*Rhodococcus erythropolis*	$1x10^7$	0.5:0.5	3	7
PFC15	Pressure Flow Column	3g/L nutrients + cement. reagent	*Micrococcus luteus*	$1x10^7$	0.5:0.5	3	7
PFC16	Pressure Flow Column	3g/L nutrients + cement. reagent	*Lysinibacillus fusiformis*	$1x10^7$	0.5:0.5	3	7
EK1	Electrokinetics (control)	3g/L nutrients + cement. reagent	N/A	N/A	N/A	14	1
EK2	Electrokinetics	3g/L nutrients + cement. reagent	*Bacillus licheniformis*	$1x10^8$	1:1	14	1

3 Results

3.1 Microbiological analysis

Selection of candidate bacteria was based on the growth criteria explained in section 2.2 (ability to grow and survive at low to medium temperatures and pH values of 4.5-10) and urease enzyme production ability according to shortlisting criteria explained in 2.3.1. According to these, the four best indigenous ureolytic bacterial strain candidates for biocementation, were *Bacillus licheniformis, Rhodococcus erythropolis, Micrococcus luteus, and Lysinibacillus fusiformis*. According to American Type Culture Collection (ATCC) all these four strains were of Biosafety Level (BSL) 1, which designates microbes that are not known to consistently cause disease in healthy adults and present minimal potential hazard to laboratorians and the environment (based on the U.S. Department of Health and Human Services, CDC/NIH Guidelines, 2007). First, two different sets of UCS tests were performed on the soils inoculated with the different monocultures varying the bacteria populations (assessed based on optical density measurements) and cementing agent concentrations (PFC9-16) after which, *Bacillus licheniformis* was selected for further testing based on the UCS results of the first two sets of testing shown in section 3.2 below but also, for the following additional reasons: (a) the bacterium is widespread in nature and can be found in abundance in natural soils; (b) it is motile (using its flagellum) but at the same time its elongated rod-shaped cell makes it difficult to flush out during pressure or EK injection; (c) it is a relatively small bacillus (about 1 μm in diameter, against about 2 pm for *B. cereus,* Bisset and Street, 1973) which facilitates its motility through smaller pore throats (d) it is reported to be facultative anaerobic (e.g. Clements et al, 2002) so it can survive in environmental conditions of reduced oxygen supply (e) it is a spore generating bacterium: this feature could be exploited for potential self-healing mechanisms (Petrova-Botusharova, 2017).

3.2 UCS results and chemical analysis of UCS samples

3.2.1 Pressure flow column results

Figure 4 shows detailed UCS results for the different treatments and implementation methods (see Table 2). Together with the UCS results other parameters of relevance are also plotted, to assist in the interpretation of the UCS testing results, namely water content of the samples at the end of the testing (which could affect the strength of the soil), $CaCO_3$ content based on acid digestion testing (which would show that biocement has been produced), ammonia concentration linked to the progress of the urea hydrolysis reactions (1-4) and which should be monitored as it is an undesirable reaction by-product (see Discussion section) and pH at the end of the test. The strength changes compared to the respective control samples with the nutrients only are also summarised in Table 3 for clarity.

Fig 4.Pressure flow column results: (a) unconfined compressive strength, q_u (kPa); (b) $CaCO_3$ content (% w/w) (end of test); (c) Moisture content (% w/w of dry soil) (end of test); (d) maximum NH_4^+ concentration (mg/L); (e) end-of-test pH

Table 3: Summary of unconfined compressive strength changes of treated samples

Pressure flow column tests					
Average UCS strength, q_u (kPa) and (%) strength change compared to nutrient only solution PFC3 **(**average q_u **=**313.43 kPa) t-test p-value results refer to comparisons of the mean q_u value of each bioaugmentation treatment dataset and that of control dataset					
Strain	**Bioaugmentation (PFC3-6)**	**Bioaugm+0:25M reagents (PFC7)**	**Bioaugm+0:5M reagents(PFC13-16)**	**Bioaugm+0:75M reagents(PFC8)**	**Bioaugm+1M reagents(PFC9-12)**
Bacillus licheniformis	q_u =236.43 kPa	q_u =340.92 kPa	q_u =381.97 kPa	q_u =428.43 kPa	q_u =351.93 kPa
	q_u change = -24.6 %	q_u change = 8.78 %	q_u change = 21.9 %	q_u change = 36.7 %	q_u change = 12.29 %
	p-value = 0.008478	p-value = 0.1462	p-value = 0.01146	p-value = 0.004065	p-value = 0.09231
Rhodococcus erythropolis	q_u =256.85 kPa	_	q_u =348.75 kPa	_	q_u =327.21 kPa
	q_u change = -18.0 %	_	q_u change = 11.28 %	_	q_u change = 4.41 %
	p-value = 0.0211	_	p-value = 0.07646	_	p-value = 0.4322
Micrococcus luteus	q_u =275.49 kPa	_	q_u =364.785 kPa	_	q_u =363.12 kPa
	q_u change = -12.1 %	_	q_u change = 16.4 %	_	q_u change = 15.87 %
	p-value = 0.06583	_	p-value = 0.02917	_	p-value = 0.0331
Lysinibacillus fusiformis	q_u =325.53 kPa	_	q_u =367.37 kPa	_	q_u =363.57 kPa
	q_u change = +3.9 %	_	q_u change = 17.22 %	_	q_u change = 16.01 %
	p-value = 0.4989	_	p-value = 0.03885	_	p-value = 0.04562

Electrokinetic tests				
Average UCS strength q_u (kPa) and (%) strength change compared to respective average UCS strength of pressure flow column test t-test p-value results refer to comparisons of the mean q_u value of each EK dataset and that of the respective pressure flow dataset				
	Right electrode	Middle of sample	Left electrode	Pressure flow column
Nutrients only (EK1)	q_u =378.37 kPa	q_u =319.89 kPa	q_u **=**355.45 kPa	q_u **=**313.43 kPa
	q_u change = 20.7 %	q_u change = 2.1 %	q_u change = 13.4 %	N/A
	p-value = 0.01905	p-value = 0.6402	p-value = 0.08085	N/A
Full treatment (EK2) *Bacillus licheniformis* **Bioaugm+ 1M reagents**	q_u =457.95 kPa	q_u =411.51 kPa	q_u =448.46 kPa	q_u =351.93 kPa
	q_u change = 30.1 %	q_u change = 16.7 %	q_u change = 27.4 %	N/A
	p-value = 0.01083	p-value = 0.03075	p-value = 0.01232	N/A

A small strength increase (by 8 %) was noted with the added water flowing out of the sample (PFC1) compared to the natural sample possibly to some density increase/ consolidation effect. Comparing PFC2 (nutrient broth only) to PFC1 a 67.1 % increase in strength was observed although the moisture contents of the two specimens were very close (around 64 %). This could be attributed to the broth composition which could lead to particle flocculation/binding (e.g. salts of which NaCl have been used for soil stabilisation by ionic charge manipulation and as a group tend to flocculate soil particles and affect the strength –depending on concentrations increase in strengths have been reported in NaCl-treated soils, see e.g. Brandon et al 2009). Conversely the addition of bacteria in the nutrient broth (without cementing reagents) led to a drop in the strength in most cases but one (where the water content was lower leading to a certain strength gain); this is possibly linked to the increase in organic biomass in the soil.

All treatment solutions with bacteria and cementing reagents caused strength increases to one or another extent, compared to the nutrient broth only; overall the highest observed strains were those of samples inoculated with *Bacillus licheniformis* (CP8 and CP13), which were also found to be statistically significant at a 95% confidence level (p-value < 0.05) based on t-test results (see Table 3). These are samples treated with solutions 364 of molarities lower than 1M. The higher strengths of these samples are consistent with observations by other researchers using other bacteria e.g. Al Qabany (2013) or Ng et al (2014). Explanations offered for this were urease activity inhibition at higher calcium chloride concentrations (Whiffin, 2004) and the faster $CaCO_3$ precipitation induced by higher cementation solution leading to randomly formed crystals as opposed to more homogeneous distributions at lower cementation solution concentrations (Mujah et al, 2016). Similarly for the second and third best strengths obtained from samples inoculated respectively with *Lysinibacillus fusiformis* and *Micrococcus luteus,* the samples with reagent solutions of 0.5 M molarities performed better than those of 1 M molarities (t-tests on all these datasets showed that the differences compared to the control samples with nutrient broth were likely to be significant at a 95% confidence level). On the other hand, the 1 M reagent solution samples inoculated with *Micrococcus luteus* and *Lysinibacillus fusiformis* showed higher strengths than the respective 1 M reagent solution samples of *Bacillus licheniformis*. It is therefore possible that other treatment combinations resulting in optimised treatment composition for each bacterium could have given similar or better results than *Bacillus licheniformis*. However for the purposes of this study and in view of the limited amount of in situ soil *Bacillus licheniformis* was selected for further testing the reasons explained in section 3.1. *Rhodococcus erythropolis* appears to perform less well than the other strains, and not significant strength increases. The above strength increases were also generally reflected in the respective $CaCO_3$ contents of the samples (Fig 4b). For clarity separate plots of strength increase vs percent calcite content were made (Fig 6a). Calcite contents were generally higher for the higher strength increases compared to the control sample, as expected (some anomalous points were however noted); the highest calcite contents of an average of 1.25% correspond to an average increase in strength of 110 kPa (see Fig 4a and 6a respectively), from samples inoculated with *Bacillus licheniformis* and reagent solution of 0.75 M concentration. The relationship between calcite content and increase in strength is soil dependent. It is therefore difficult to draw direct comparisons with the literature concerning mostly biocementation of sands, especially as organic matter can inhibit $CaCO_3$ precipitation and crystal growth (Lebron and Suarez, 1996 and 1998). Indicatively however we can mention that Duraisamy (2016) recorded unconfined compressive strengths between 120-200 kPa for calcite contents between 0.8%-1.33%. Figure 7 (a) and (b) shows respectively indicative SEM-EDS results from PFC8 (*Bacillus licheniformis*, 0.75 M reagent solution) for which 1.25% $CaCO_3$ was determined and from PFC16 (*Lysinibacillus fusiformis*, 0.5 M reagent solution for which 0.9 % $CaCO_3$ was determined); well distributed precipitation products have formed on the particles; indicative EDS spectra from sites on the sample show clear Ca and C peaks concurring to $CaCO_3$ precipitation. There is some small water content decrease in the treated samples which could have somewhat affected the strength (Fig 4c) but this would expected to be small as water content variations (especially across the bioaugmented samples) are generally small.

Figure 4(d) represents the maximum measured NH_4^+ concentrations (mg/L) (which are related to urea hydrolysis as described in reactions (1)-(3)). Generally, lower reagent concentrations led higher NH_4^+ concentrations which could be explained by the observation by Whiffin (2004) mentioned above regarding urease activity inhibition at high calcium chloride concentrations. It should be noted that the concentrations exceed the allowable limits for total ammonia (NH3 and $NH4^+$) for drinking water according to UK legislation, set to 0.5mg/L (The Water Supply (Water Quality) (Amendment) Regulations, 2018).

Finally the pH changes at the end of the test (where pH drops following carbonate precipitation) compared to the natural sample were moderate to low, with the highest recorded changes (increases) occurring in the samples with 0.5 M reagent which also generated overall the highest amount of reaction products compared to the respective samples with 1 M reagent solutions (Fig 4e).

3.2.2 EK treatment results

Figure 5 shows detailed UCS results accompanied by other end of test measurements (water content, $CaCO_3$ content and pH). EK treatment (which is a method of ground improvement *per se*) considerably enhanced strengths compared to the pressure flow, even for the nutrient only sample. Most considerable strength gains (20.7-13.4 %) were recorded close to the electrodes with the addition of nutrients compared to the respective pressure flow column sample (PFC3). However, when nutrients only were added the strength in the middle of the sample only increased by 2 % compared to the pressure flow column; the increase was noted despite the slightly higher water content at the middle points of EK1 sample (i.e. 65.9 % vs 63.4 % for EK1 and PFC3 respectively). The higher strengths close to the electrodes 424 could also be affected by the lower moisture contents at these points (58.4-59 %) compared to the middle points of the sample (65.9 %). On the other hand strength gains became considerably higher with the addition of the full treatment (bacteria and cementing reagents, EK2) and significant at a 95% confidence level according to t-test results (see Table 3) even at the middle of the sample. Note also that in EK2 the water contents of the areas close to the two electrodes were consistent to that of the respective pressure flow column test (PFC9) and therefore the increase in strength compared to PFC9 cannot be partly attributed to water content effects (Fig 5c). Similarly, as the water contents at the electrodes and middle points respectively of EK1 and EK2 are almost the same the increase in strength is due to the combined EK-bacteria effects rather than moisture content effects.The differences in the strengths of the areas close to the two electrodes now reduced to less than 3%. This could be possibly attributed to the better distribution of the treatments in the soil when EK is used. The lower strength at the middle points of the EK2 sample could also be partly attributed to the higher moisture content compared to the moisture contents of the EK2 close to the electrodes.

Consistently with the increase in strength, calcite precipitation in EK2 (full treatment including bacteria and cementing reagents) increased drastically compared to column flow (101.1 % , 36.5 % and 45.9 % increase respectively at the right electrode, middle of sample and left electrode) (see Fig 5b and 6b).

As expected, EK treatment affected the pH more drastically (the process causes hydrogen ions to be generated at the anode and hydroxyl ions at the cathode, so a pH gradient develops between the electrodes). Close to the electrodes the soil was slightly acidic, i.e. not favourable for calcite precipitation but it increased considerably in the middle of the sample. There was no considerable difference in the pH between the EK1 and EK2 tests (nutrients only and bacteria+cementing reagents respectively). It is interesting that despite the polarity reversal the effects on strength and calcite precipitation remain more pronounced at the right electrode from which injection started. This is difficult to explain and requires further investigation, and so does the effect of the soil acidity next to the electrodes on the $CaCO_3$ concentrations.

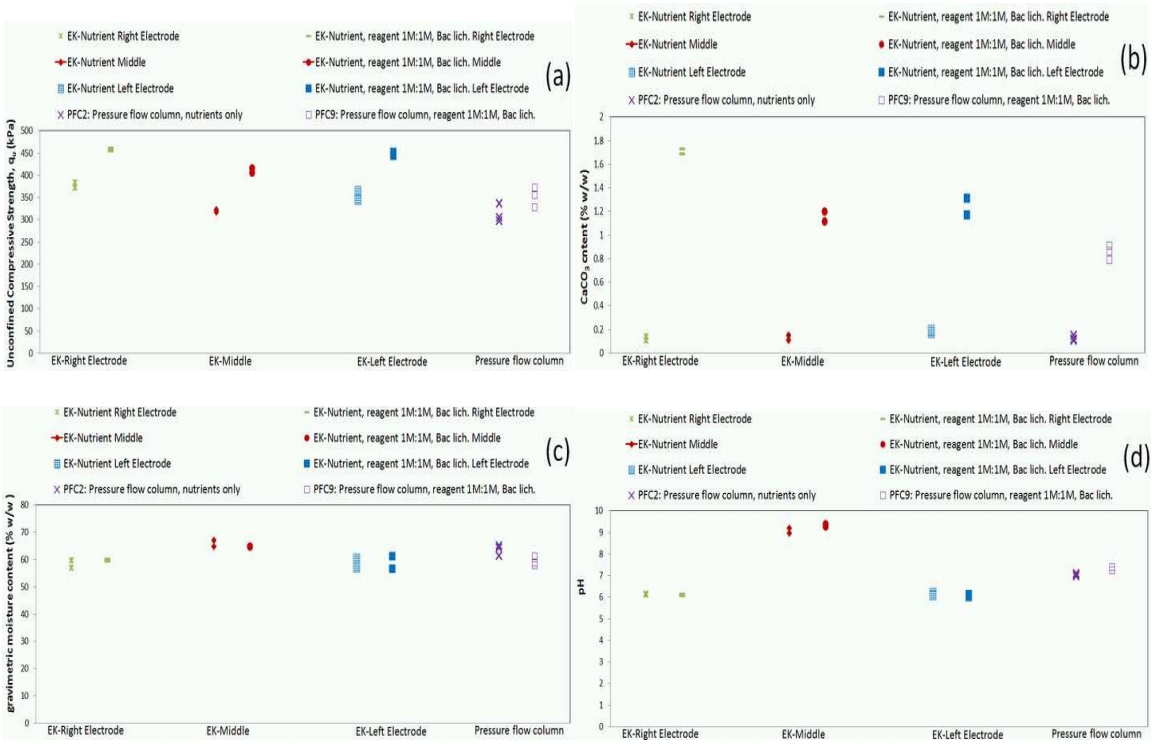

Fig. 5. EK testing results compared to respective pressure flow column results (end of test): (a) unconfined compressive strength, q_u (kPa); (b) $CaCO_3$ content (% w/w); (c) Moisture content (% w/w of dry soil); (d) end-of-test pH

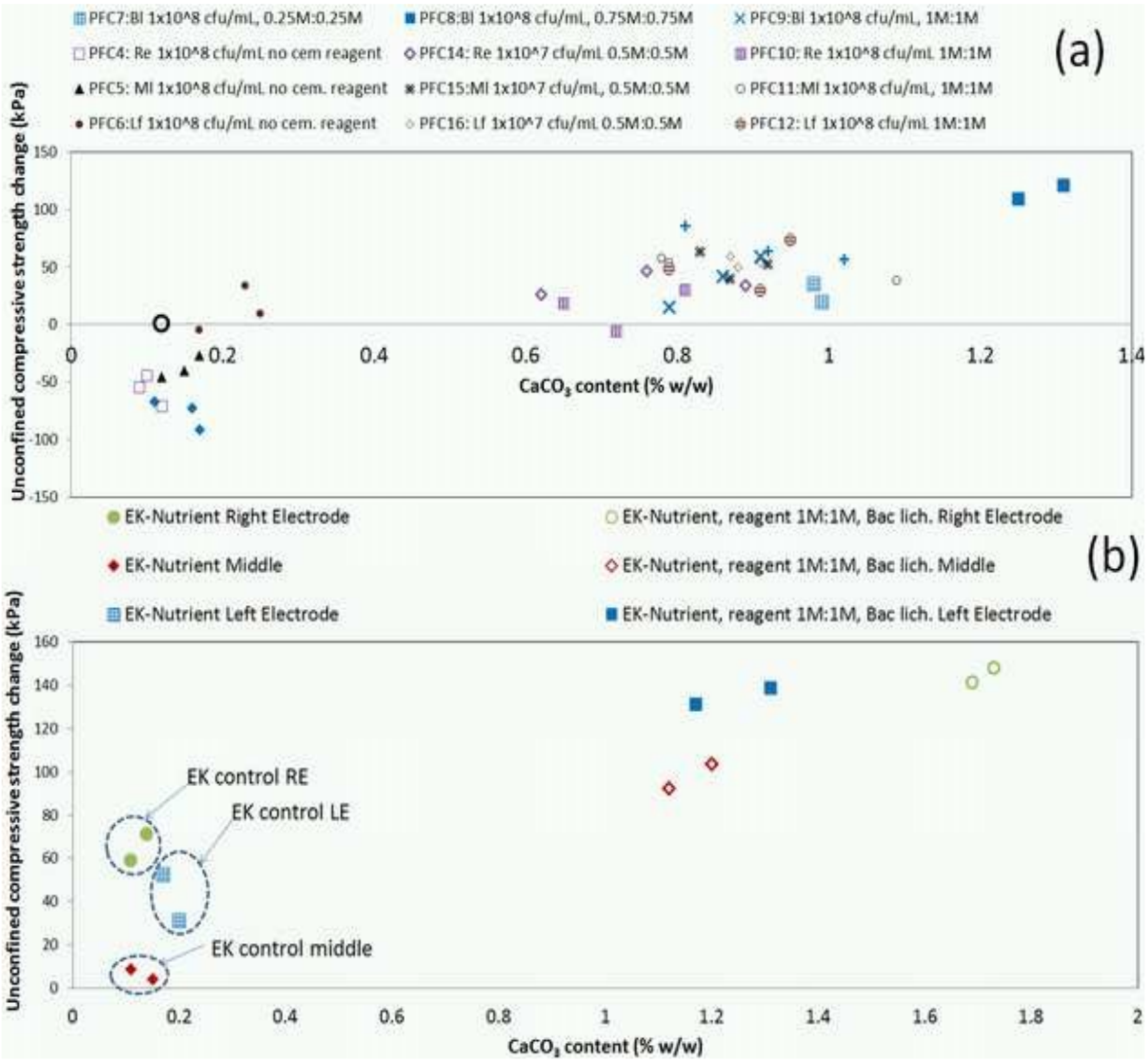

Fig 6. Unconfined compression strength changes (kPa) compared to control sample (nutrients only) vs calcite content (% w/w): (a) Pressure flow column; (b) EK

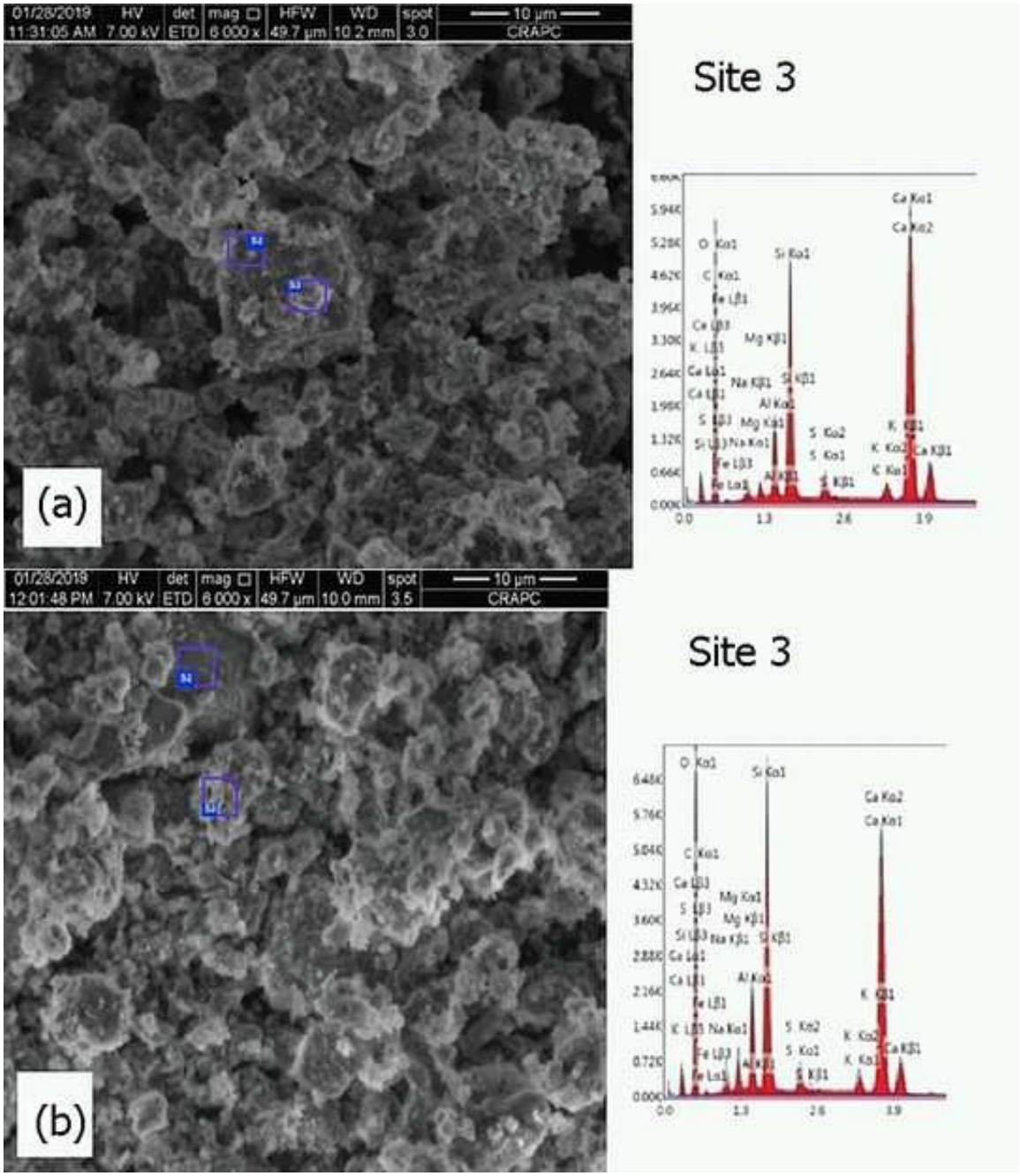

Fig.7. SEM-EDS results of selected samples: (a) PFC8 and (b) PFC16

4 Discussion

The study aimed to prove feasibility of biocementation of the Peat Fens ground; we thus focused on strength improvement and calcium carbonate production as indicators of biocementation occurring. Whilst cementation is expected to increase stiffness and it is believed that coating of the peat particles by biocement could reduce further peat oxidation, these points need to be verified. Furthermore to solve UK rail's peat foundation problems it is necessary to conduct detailed site monitoring and investigation of the different mechanisms potentially driving the embankment settlement (consolidation, secondary compression, peat oxidation, shrinkage, plastic squeezing); this is the focus

of the next stage of the research. Furthermore, in this work we studied in detail soil from one layer only (due to material limitations); however our analysis of borehole data showed also some peat layers of different state with very high water and organic matter that will require separate study.

In this work ureolytic bacteria were isolated as the first biocementation candidates to use. This was because calcite precipitation through urea hydrolysis is considered a most straightforward and the most easily controlled mechanism of MICP (Duraisamy, 2016) as well as because ureolytic bacteria are found in abundance in soils. However the process releases to the environment major undesirable by-products with potential adverse impacts on human health and environment (ammonia, ammonium ions and salts: for instance, using $CaCl_2$ as source of calcium ions ammonium chloride NH_4Cl, a recognized groundwater contaminant is produced).

Ammonia is one of the criteria air pollutants according to EU regulations; ammonium ion and total ammonia (NH_3 and NH_4^+) concentration in drinking water is also regulated. NH_4^+ ions can cause acidification of ground and water bodies, which can harm plant and animal life they and are very toxic to aquatic organisms (Keyka et al 2018). For real-scale engineering works facilities for removal of ammonia from the air and ammonium ions from effluent are required, resulting in increased costs, complications (e.g. obtaining permits from regulatory bodies) and other issues with sustainability of the process (e.g. use of large amounts of water for flushing the effluent to reduce ammonia concentration, which has been adopted in practice). The success of in situ bio-cementation using ureolytic bacteria will thus 498 depend on finding effective and sustainable approaches to reduce these impacts; this could be particularly challenging for large scale applications such as linear transport infrastructure.

Feasibility of large scale in situ applications would also need a thorough consideration of other economical and legislative aspects, for instance the cost of substrates (the use of waste products has been proposed as a way to reduce these, e.g. van Paassen et al. 2009) and regulatory and public acceptance issues related to the use of microorganisms. The proposed use of indigenous microorganisms, which are thriving in their natural environment (hence adaptability issues would not be of concern) could be more cost effective; it circumvents regulatory issues related to packaging and transport of biological material and is meant to reduce the impact on the local ecology. A study of the species distribution in the treated soil samples would also be required to assess whether the bioaugmented strains were the predominant ones or whether the supply of nutrients caused other bacteria (including pathogens) to overgrow the added ones. Finally, another aspect related to the sustainability of the process is the durability of $CaCO_3$ as cementing agent; this has been queried, due to possible dissolution in acidic environment (e.g. due to acidic groundwater). However dissolution rate is expected to follow similar rates as for naturally formed $CaCO_3$ which is a very slow process (van Paassen, 2009). In addition, Peat Fens environment is normally alkaline (Hobbs, 1986). If required renewal of the treatment is possible in particular if self-healing mechanisms of spore generating bacteria can be exploited (this is a process worth researching further); if EK is used for implementation, electrodes could remain in the ground as reinforcing or draining elements (Nettleton et al 2018), thus ready to reuse for treatment renewal, if required.

This latter point links to the major challenge of finding suitable ways to implement nutrients and bacteria (if bioaugmentation is used) under existing infrastructure. As mentioned earlier in this study bacteria were premixed in the first stage of the research to prove biocementation of this type of soil circumventing complications related to bacteria delivery. However mixing is unfeasible under existing infrastructure unless delivered in the form of deep mixing. The latter technique was avoided by other researchers due to concerns of bacteria viability under the stresses associated with industrial mixing processes; however recent laboratory work by Duraisamy (2016) applying deep mixing processes using gram-positive bacteria (as in our case) showed promising results; gram-positive bacteria have thick 528 and rigid cell walls which may potentially protect them from puncture or lysis when external forces are applied during mixing. Furthermore our results showed promise for EK implementation: EK enhanced the strength of the biocemented soil compared to pressure driven flow; more importantly EK can convey the treatments under the existing embankments without pore pressure development and with the potential of not affecting groundwater table levels (which could trigger peat oxidation). Unlike pressure-driven flows in which preferential fluid flows are inevitable (leading to inhomogeneity of treatment), EK has the potential to give a more uniform flow distribution and a higher degree of control over the direction of the flow; especially promising is the polarity reversal during treatments. A disadvantage is the considerable expenditure coming from the required electric power sources and energy consumption; the use of renewable energy sources has been suggested to reduce such costs (Hassan et al 2016) but this would require further research at field scale. Nevertheless some recent case studies of cost savings and reduced carbon footprint were reported when using EK (without bacteria) compared to other soil stabilisation techniques (soil nailing) (Nettleton et al 2018). Other possible issues could arise from poor electrical connection, temperature increase in the soil temperature with consequent soil desiccation (reducing EK efficiency but also potentially exacerbating settlements) durability and effect of electrode material on bacteria, and other effects of the process on bacteria (electric current intensity, duration and intermittence, pH gradients and increased soil temperature). The very limited examples of application at field scale of combined EK and biological methods means that further pilot field testing is urgently needed towards optimisation of the combined processes before this can become a viable in situ technique. In this respect an advantage of the bacteria used in this study can potentially tolerate pH ranging from 2-10 and as well as increased soil temperature next to electrodes as they are proven to grow at temperatures up to 50-55 °C (Ronimus et al, 1997).

5 Conclusions

The aim of this work was to assess the feasibility of using indigenous bacteria to biocement Peat Fens soil samples based on unconfined compressive strength (UCS) and $CaCO_3$ precipitation measurements. To this effect non-pathogenic indigenous ureolytic microbial candidates were screened and isolated. Four BSL1 strains (ATCC c 558 lassification) were selected as the most suitable candidates, based on their growth rate, urease activity and their viability in a wide range of temperatures, pH and soil moisture contents corresponding to typical seasonal field conditions;

namely *Bacillus licheniformis, Rhodococcus erythropolis, Micrococcus luteus,* and *Lysinibacillus fusiformis.* Natural non-sterile soil was inoculated with the different monocultures by mixing and nutrients implemented using two different methods, i.e. pressure flow and EK. The first set of UCS results showed samples inoculated with *Bacillus licheniformis* to have higher strengths compared to those inoculated with other monocultures. Further parametric studies were therefore performed with *Bacillus licheniformis* monoculture and equimolar solutions of $CaCl_2$ and urea of different molarities implemented by pressure flow: it was shown that 0.5M and 0.75M solutions resulted in higher strengths compared to 1M solutions which is consistent with literature findings for other bacteria (e.g. *Sporosarcina pasteurii*) and could be linked to urease activity inhibition at higher calcium chloride concentrations. Consistently with the strength increase, $CaCO_3$ concentrations in the treated soils also increased; highest $CaCO_3$ contents recorded were in the region of ca. 0.9-1.3% corresponding to samples showing up to 37% higher strengths compared to the control samples. Potential effects of water contents on strength were assessed and excluded as samples maintained similar water contents throughout treatment. EK implementation of one selected treatment further enhanced treated soil strength resulting to up to 30% increase in strength compared to pressure flow.

The above results in terms of strength and $CaCO_3$ formation are encouraging as to the feasibility of biocementing Peat Fens foundation soil using indigenous bacteria. It is believed that this is a positive advancement compared to using exogenous species as the problem of adaptability to site conditions (affecting exogenous microorganisms) is largely overcome.

The implications of these results are of particular interest for linear infrastructure owners, who are seeking new, cost effective and potentially more sustainable ground improvement techniques, of paramount importance for this type of works. For this, upscaling and proving the techniques in field pilots towards is of paramount importance for adoption by industry. EK, which has enhanced the strength, shows promise as a suitable technique to treat Peat Fens soil under existing embankments. Ultimately however the selection of most adequate process for field implementation will strongly depend on considerations of efficiency, 588 undesired by-products and energy consumption, at real scale in situ. Nevertheless, although a number of challenges lie ahead towards successful field implementation for this application, the active involvement and interest of railway owners willing to facilitate pilot field applications is the way forward to address and resolve related issues, thus making actual progress in translating this promising technology from the laboratory to the field.

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